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Observations and measurements of planktonic bioluminescence in and around a milky sea

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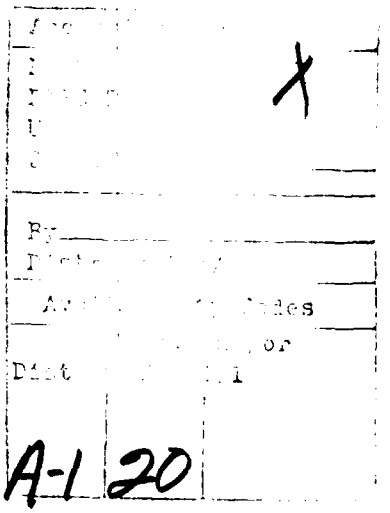
Abstract: The Indian Ocean and Arabian Sea have been observed to exhibit surface bioluminescent displays unparalleled in intensity and spatial extent. In July 1985, we conducted bioluminescence measurements in the surface waters of the western Arabian Sea during the period of the southwest monsoon and to identify the causative plankton for these displays. While an intense stimulable bioluminescence signal was always present (range: $1 - 5 \times 10^8$ photons per second per cubic centimeter of turbulently flowing seawater, measured by an onboard underway photometer system), a unique type of bioluminescence display, known as "milky sea", was observed on the ocean surface for 3 days. Luminous dinoflagellates, zooplankton, and bacteria were isolated and tested in a shipboard laboratory photometer system for bioluminescent potential. Their light output values, together with abundance of luminous species present in collected plankton samples, indicated a stimulable bioluminescence field superimposed on a milky sea. The stimulable bioluminescence field was dominated by luminescent dinoflagellates, primarily *Pyrocystis* spp. and *Protoperidinium* spp. The zooplankton contribution to the overall light budget was estimated at $<50\%$ for the samples examined and was dominated at times by euphausiid furcilia and *Pleuromamma* spp. copepods. Luminous larvaceans, ostracods, siphonophore nectophores, and radiolarians were intermittently present in the samples. First observations of bioluminescence were recorded in the calanoid copepod *Pleuromamma quadrungulata* (Dahl) and the Calycophorae siphonophores *Chelophyes contorta* (Lens *et* Van Riemsdijk), *Abylopsis tetragona* (Otto), and *A. eschscholtzi*. *Phaeocystis* colonies glowed continuously and appeared to act as a substratum for the colonizing luminous bacteria *Vibrio harveyi*. This bacteria is hypothesized to be the source of the luminescent "milky sea."

Key words: Arabian Sea; Bioluminescence; Dinoflagellate; Measurement; Milky sea; Zooplankton



INTRODUCTION

Historically, the Indian Ocean and Arabian Sea have exhibited surface bioluminescent displays unparalleled in frequency, intensity, duration, and spatial extent. Many of these observations have been compiled in various reports (Smith, 1926, 1931; Harvey, 1952; Tarasov, 1956; Turner, 1965; Staples, 1966; Tett & Kelly, 1973; Kelly

Correspondence address: D. Lapota, Naval Ocean Systems Center, Radiation Physics Branch, Code 524, San Diego, CA 92152–5000, U.S.A.

& Tett, 1978; Herring, pers. comm.) and from records of merchant marine ships transiting the area. Disturbed water or stimulable luminescence (luminescence produced by plankton subjected to mechanical stimulation) can be observed from breaking wave crests and swimming shoals of fish. The Arabian Sea is known for a unique type of bioluminescence, referred to as "milky seas." This phenomenon is distinct from other types of bioluminescence in that the emitted light is observed as an opaque white glow of unvarying intensity that can stretch over vast areas of the ocean (horizon-to-horizon displays are not unusual for this type of phenomenon; Turner, 1965). In the marine environment, luminous bacteria are the only known organisms capable of emitting light continuously (Harvey, 1952; Nealson & Hastings, 1979; Haas, 1980; Hastings & Nealson, 1981), and they have been hypothesized to be the source of luminescence of milky seas (Turner, 1965; Tett & Kelly, 1973). Of the 87 reports compiled by Turner (1965) describing milky seas, 77 were located in the northern Indian Ocean, particularly in the Gulf of Aden and off the southern Arabian coast during months of the southwest monsoon (July, August, and September). Regretfully, biological sampling was not undertaken during any of these sightings to identify the causative plankton.

In July 1985, while completing a set of bioluminescence measurements during the southwest monsoon, we observed a milky sea display in the western Arabian Sea and collected plankton samples in an attempt to identify the major sources of the bioluminescence.

METHODS

The cruise track of the study area (Fig. 1) ran along the edge of the western Indian Ocean from Mombasa, Kenya, up to the Horn of Africa, back south east off Somalia and the island of Socotra, and then northeast to Karachi, Pakistan.

SEAWATER COMPLETE AGAR MEDIUM (SWC)

On the chance that we might encounter a milky sea and to test the hypothesis that the display might be bacterial in origin, we prepared seawater complete agar medium (SWC) on a regular basis, sampling fresh seawater from an onboard system, filtering to 20 μm to exclude the larger plankters, and plating serial dilutions (full strength, 1 : 10, 1 : 100, and 1 : 1000) of the seawater samples. SWC medium, its preparation, and methods of isolation of luminous colonies have been described (Nealson, 1978). When luminous colonies were observed, they were isolated in a dark room under red light and transferred with sterile toothpicks into agar storage vials (Nealson, 1978). The bacteria were identified by plating the bacteria on different C sources and observing growth. In all, 19 characters were used as criteria for their identification: growth at 4 °C, growth at 35 °C, amylase, lipase, gelatinase, growth on maltose, cellobiose, gluconate,

glucuronate, mannitol, proline, lactate, pyruvate, acetate, propionate, heptanoate, D-α-alanine, L-tyrosine, and α-ketoglutarate (Reichelt & Baumann, 1973; Nealson, 1978).

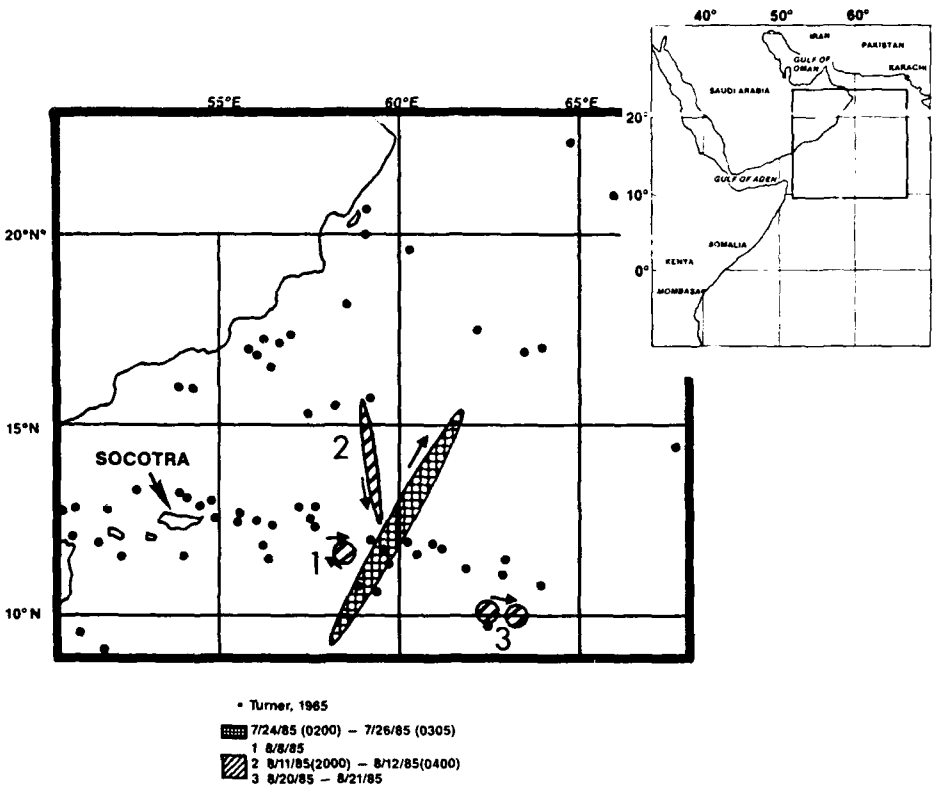


Fig. 1. Location of milky sea observed in this study and by others. Arrows indicate ship heading in Arabian Sea.

EPIFLUORESCENCE MICROSCOPY

Colonies of *Phaeocystis* were preserved in a 5% formalin-buffered seawater solution. One of these colonies, No. 112, was processed for epifluorescence microscopy for bacteria enumeration using the DAPI (4′6-diamidino-2-phenylindole) stain which is highly specific to DNA. The DAPI–DNA complex fluoresces bright blue (450 nm) when excited by UV (360 nm) light, but unbound DAPI fluoresces a weak yellow (James & Cope, 1978). Consequently, the nuclear DNA of bacteria can be seen under microscopy against a dark background when illuminated by UV. Luminous bacteria, however, cannot be distinguished from the general bacterial population by this method. Standard procedures (Hobbie *et al.*, 1977); James & Cope, 1978; Porter & Feig, 1980) were employed and modified for use with a single colony. The specimen was stained in a

(0.2-μg · ml^{-1}) DAPI solution prepared from frozen stock and sterile (0.22-μm filtered) distilled water. The colony was placed on a Nucleopore polycarbonate (0.2-μm) filter stained black to increase its contrast with the fluorescent bacteria, and made into a microscope slide. It was photographed using a 35-mm camera mounted on a Zeiss universal fluorescent microscope with a 100-W Hg illuminator, an UV (G 365) filter combination, and a 100 × neofluar oil-immersion objective.

BIOLUMINESCENCE MEASUREMENTS

Surface-water bioluminescence

Surface-water bioluminescence was measured continuously during the entire cruise (9–28 July 1985, USNS Wilkes T-AGS 33), with an onboard underway photometer system from seawater collected continuously from beneath the hull of the ship (3 m below sea surface) (Lapota & Losee, 1984; Losee *et al.*, 1985) (Fig. 2). The seawater either was discharged overboard or into a series of plankton-collection tubs for concentration and later identification of the light-emitting plankton. The turbulence generated within the chamber acted as a constant source of stimulation for light production in the plankton. The bioluminescence signals were summed for 100 s with scalers from two RCA 8575 photomultiplier tubes, one PMT measuring the full spectrum of the bioluminescence signal ($\approx$380–620 nm) while the other PMT measured UV bioluminescence (at wavelengths $<$400 nm by inserting a Schott 2.5 mm thick Ug-11 filter between the viewing chamber and the PMT) to give an average measurement of bioluminescence intensity as photons per second per cubic centimeter of seawater. This system and the laboratory plankton test chamber were calibrated in the laboratory with a solution of the bioluminescent bacterium *Photobacterium phosphoreum* with details published elsewhere (Losee *et al.*, 1985). Simultaneous measurements of surface-

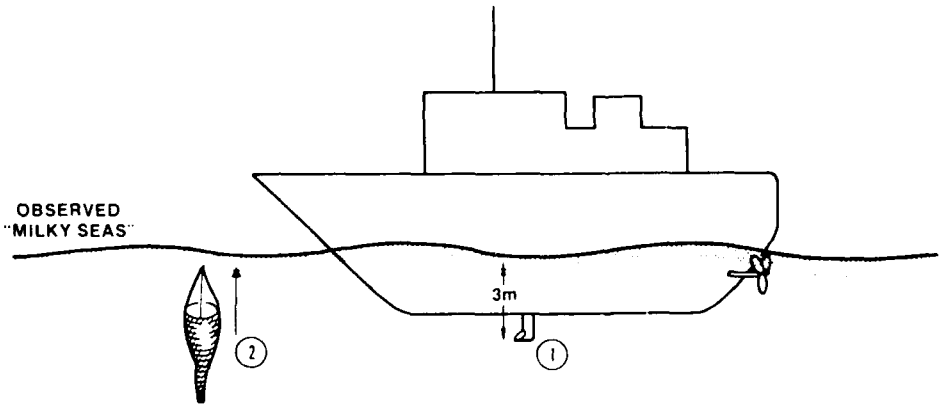


Fig. 2. Seawater sampling schemes used for onboard photometer system and collecting luminous plankton in this study: (1) sea-chest intake 3 m below sea surface, and (2) 53-μm plankton net pulled up from below and through sea surface collecting surface populations.

seawater temperature (Sea Bird SBE-3) and chlorophyll fluorescence (Turner Designs 10–005R) were also measured.

Laboratory testing system

Another system, the laboratory plankton test chamber (LPTC), measured light output from individually isolated dinoflagellate cells and zooplankters collected from plankton net tows. The plankters were held individually in vials filled with filtered seawater (0.45 μm) and maintained either in a flowing filtered seawater bath (0.45 μm) or kept in a shipboard refrigerator at 18 °C prior to testing to permit recovery of the plankters' bioluminescent potential. The plankters were stimulated to luminesce when the water in which they were maintained was removed by a vacuum pump. The organism's flash was viewed by a pair of photomultiplier tubes with the signal intensity displayed on multichannel analysers operating in the multiscaler mode. The details of both the onboard underway system and the LPTC systems have been published (Lapota & Losee, 1984).

BIOLOGICAL SAMPLING, IDENTIFICATION, AND ENUMERATION OF PLANKTON

Plankton samples were collected from the discharge of the onboard underway photometer system every 4 h along the entire cruise track. Forty liters of seawater were pumped into plankton-collection tubs, filtered through a 20-μm collection cup, and preserved in a 5% formalin buffered solution. Plankton samples were collected from before the milky sea area (Samples 80–95), in the milky sea (Samples 96–111), and beyond the milky sea (Samples 112–117) and microscopically examined for differences in numbers and species of phytoplankton and zooplankton. In all, 10 ml of each of the 12 concentrated samples (40-l samples concentrated to 100 + 15 ml) were settled in an Ütermohl chamber with rose bengal stain overnight and counted at 100 × for diatoms, dinoflagellates, and other microplankton. Diatoms were estimated by examining one or more fields of view. Each of the 40-l samples was examined for all the larger and more infrequent zooplankters missed by subsampling. The numbers of dinoflagellates and zooplankters found in the pumped samples have been extrapolated to numbers $\cdot m^{-3}$. Other plankton collections were made with sea-surface drifting plankton nets (5–0 m, 53-μm mesh net, and collection cup) and from deeper vertical tows (400–0 m, 500-μm mesh net, and collection cup). The surface tows were completed every day 1 h prior to sunset for sorting, isolating, and loading single cells into the LPTC sample vials. The deeper vertical tows were attempted every night of the cruise 3–4 h after sunset. Taxonomic authorities used were diatoms: Cupp (1943); dinoflagellates: Taylor (1976); tintinnids: Kofoid & Campbell (1929); copepods: Brodsky (1950); euphausiids: Boden *et al.* (1956); siphonophores: Totton & Bargmann (1965); radiolarians: Anderson (1983); appendicularia: Bückmann & Capp (1975).

DATA COLLECTION AND ANALYSIS

The data are displayed as species contribution per liter of seawater for diatoms, dinoflagellates, copepods in samples collected before, during, and after the milky sea. The percent contribution by luminous dinoflagellates and zooplankton to the estimated light output (ELO) per liter of seawater for plankton samples are discussed. A light budget for the luminous plankton collected from the onboard underway system and from plankton net tows was calculated based on average light output values recorded from individual bioluminescent plankton. A comparison of the summed estimated light output (SELO), photons, from all luminescent plankton per liter of seawater and the bioluminescence signal rate, photons per second per cubic centimeter of seawater, from the onboard photometer system was made. A diel bioluminescence activity factor also has been included in the LPTC plot for those samples collected during the hours of transition and daylight, a period of time when bioluminescence activity decreased from its maximum near midnight. The summed estimated light output from plankton per liter of seawater was treated with this factor for samples collected at 1000 (No. 91, reduced by a factor of 4), 0315 (No. 111, reduced by a factor of 2.9), and 0600 (No. 112, reduced by a factor of 2.5) to mimic the measured natural diel nature in the bioluminescence signal at that particular time of the cycle. This factor was estimated from the preceding

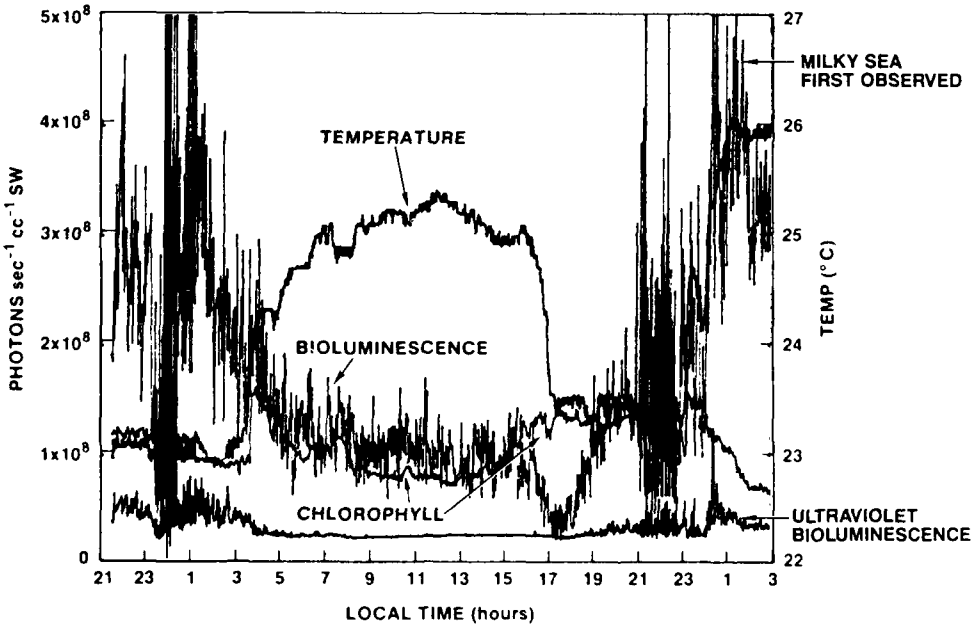


Fig. 3. Continuous measurements of sea-surface water temperature, bioluminescence intensity, UV bioluminescence, and Chl *a* fluorescence as ship transited through edge of first night's (24 July, 1985) observation of milky sea. Bioluminescence intensity is plotted as photons per second per cubic centimeter of seawater. Chl *a* is plotted as relative fluorescence (position: 9°42.749'N; 58°01.463'E).

maximum bioluminescence activity (midnight) to the time the transition and daylight samples were collected.

RESULTS

MILKY SEA OBSERVATIONS

A milky sea first was observed on 24 July 1985 at ≈0200 from the ship's bridge at 9°42.7′N : 58°01.4′E (Fig. 1). This initial observation occurred 2 h following moonset and after the ship crossed the edge of warmer water in which the sea-surface temperature increased from 23.1 °C at 2300 to 25.8 °C at 0110 (Fig. 3). Prior to entering this area, sea-state conditions were classified as Beaufort 6–7 with near-gale force winds being in excess of 28 kn. Wind direction was from 220 (true), and sea spray from breaking waves was quite apparent. In addition, a milky fog above the sea surface (≈ 10 m) was observed while visibility from the bridge was ≈ 10 miles. The milky sea was not visually apparent by sunrise (0601), but was observed again the nights of 25 and 26 July and ended abruptly at 0305 on 26 July. The sea-surface appearance changed from white to black water as if a barrier had been crossed. The sea-surface temperature at the interface changed only from 25.9 to 26.1 °C (Fig. 4). During this last morning's observations,

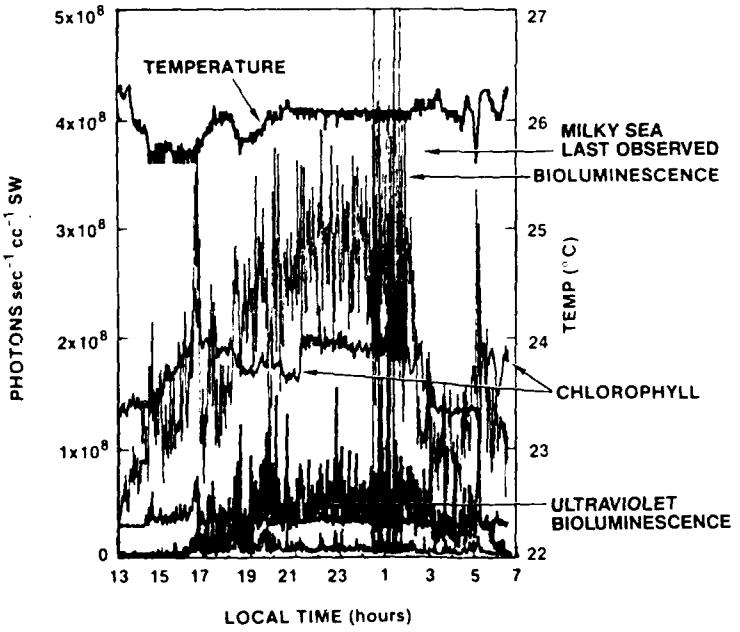


Fig. 4. Continuous measurements of sea-surface water temperature, bioluminescence intensity, UV bioluminescence, and Chl *a* fluorescence as ship passed through last night's (26 July 1985) observation of milky sea. Bioluminescence intensity is plotted as photons per second per cubic centimeter of seawater. Chl *a* is plotted as relative fluorescence (position: 15°18.310′N : 61°28.385′E).

winds varied in intensity from 24 to 26 kn with sea-state conditions calmer than expected considering the prevailing wind conditions. The fog previously noted was not apparent in these calmer seas. In this last display, dark stratocumulus clouds along the horizon contrasted sharply with the milky-white sea. The display extended from horizon to horizon in all directions with ≈ 10-mi visibility. A bioluminescent wake generated by the ship while underway and from the ship's bow thruster while on station was significantly brighter than the surrounding glowing milky sea.

Other reports of milky seas from the same area and time frame were reported to the U.S. Naval Oceanographic Office from other transiting ships. Their positions and times of observations have been included in Fig. 1. ("1" – 8 August 1985; "2" – 11 August 1985; "3" – 20 August 1985). Historical records of this phenomenon listed in an earlier report are marked in filled circles throughout the study area (Turner, 1965; also Fig. 1).

BIOLUMINESCENCE INTENSITY MEASUREMENTS

The underway bioluminescence data exhibited a marked diel variation throughout this area and the entire cruise. Maximum intensities were measured near midnight with minimum levels recorded during midday (Fig. 5). The transition period (building to maximum intensity) usually occurred between 1700 and 2100 while the start of the

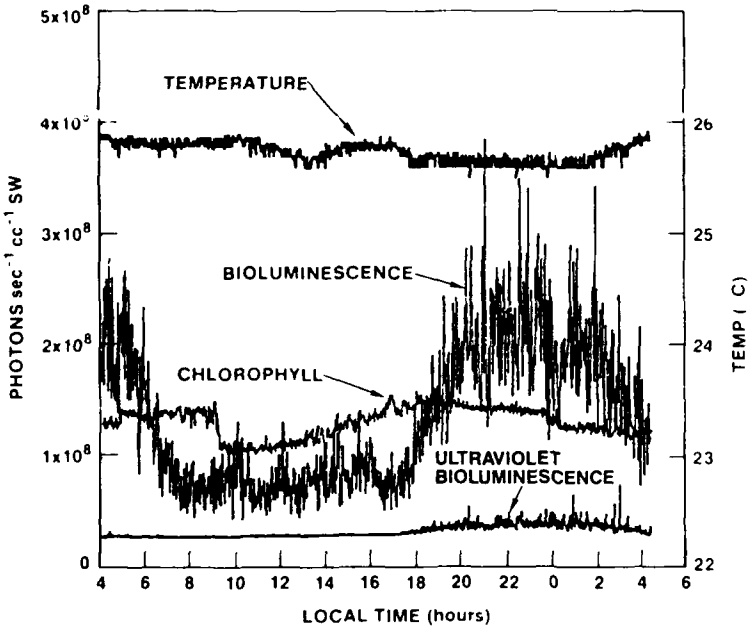


Fig. 5. Continuous measurements of sea-surface water temperature, bioluminescence intensity, UV bioluminescence, and Chl *a* fluorescence. Diel pattern in stimulable bioluminescence was commonly observed throughout 20-day expedition. Bioluminescence intensity is plotted as photons per second per cubic centimeter of seawater and Chl *a* is plotted as relative fluorescence (position: 9°25′N : 55°02′E to 9°56′N : 54°41′E).

transition to minimum intensity frequently began at ≈0300 and lasted until 0800. Bioluminescence intensity was consistently high during the entire cruise with a range of $1–5 \times 10^8$ photons $\cdot s^{-1} \cdot cm^{-3}$ of seawater observed during darkness, and $4 \times 10^7–1 \times 10^8$ photons $\cdot s^{-1} \cdot cm^{-3}$ measured during daytime hours. The dark current of the system throughout the cruise was measured at ≈500 counts $\cdot s^{-1}$, which is significantly less than the measured stimulable bioluminescence minimum–maximum range of $8 \times 10^5 – 1 \times 10^7$ photomultiplier (PMT) counts $\cdot s^{-1}$ for day to night intensities, respectively.

BIOLOGICAL SAMPLING

Seawater collected from the onboard system was used to inoculate SWC agar plates. Serial dilutions ranged from full to 1 : 1000 strength. No luminous colonies were observed after several dark room examinations 24 and 96 h following inoculation. The seawater filtrate collected at 3 m in the milky sea yielded no detectable glow when measured in the LPTC. Examination of the 40-l seawater samples collected during this series did not indicate an increase in plankton abundance or any bioluminescent

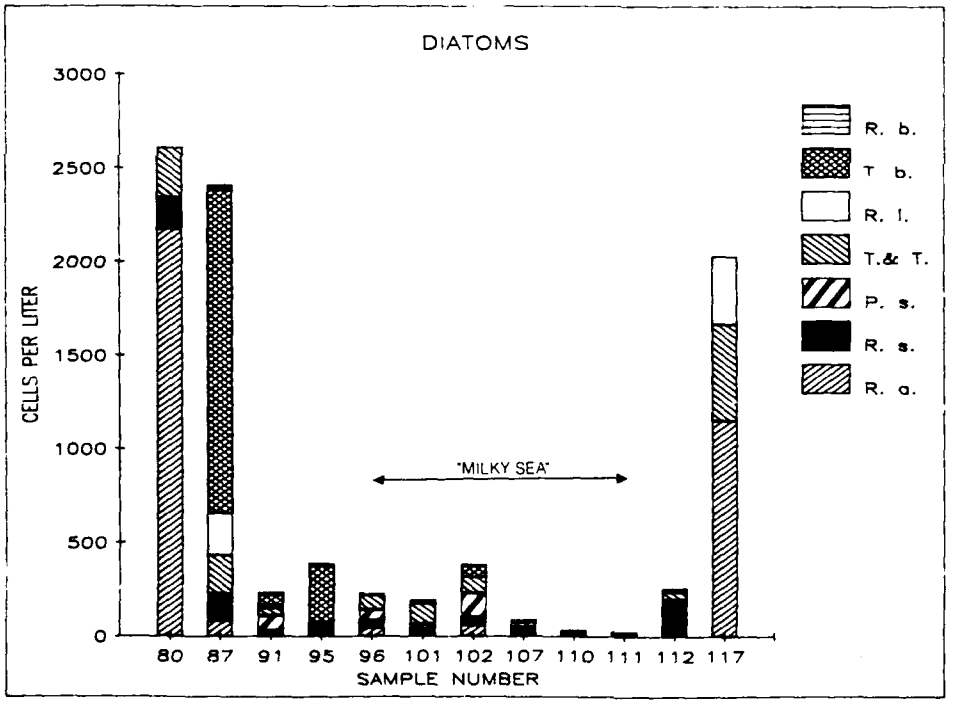


Fig. 6. Total diatom cells and predominant species contribution per liter of seawater per sample before, during, and after observed milky sea (R.a. = *Rhizosolenia alata*; R.s. = *R. styliformis*; P.s. = *Planktoniella sol*; T.& T. = *Thalassionema & Thalassiothrix*; R.i. = *R. imbricata* Brightwell; T.b. = *Thalassionema bacillaris*; and R.b. = *R. bergonii* H. Péragallo).

plankton when compared with samples collected before the appearance of the milky sea. The numbers of diatom cells decreased in the milky sea (Fig. 6), as did total and bioluminescent dinoflagellate cells (Fig. 7), and tintinnid and crustacean zooplankton numbers (Fig. 8).

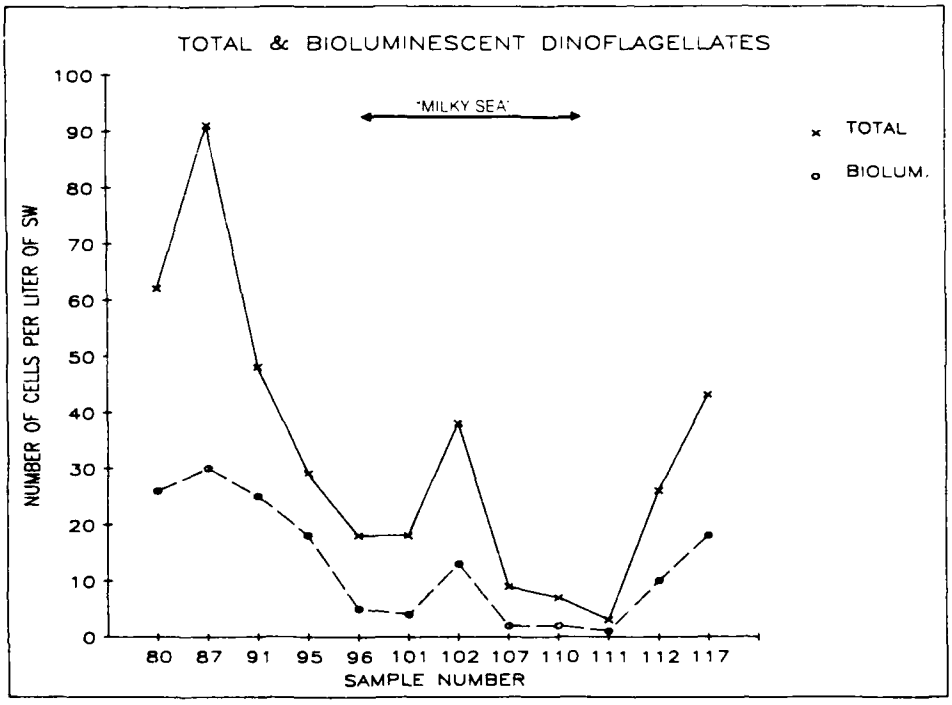


Fig. 7. Number of both total and bioluminescent dinoflagellate cells found per liter of seawater in plankton samples collected before, during, and after observed milky sea.

Samples collected through the milky sea were at times dominated by the centric diatoms *R. styliformis* Brightwell, *Planktoniella sol* (Wallich) Schutt, and the pennate diatoms *Thalassionema* and *Thalassiothrix*, while *Thalassionema bacillaris* and *R. alata* Brightwell, dominated the diatom collections before and after the milky sea (Fig. 6). Of the dinoflagellates encountered, *Ceratium*, *Protoperidinium*, and *Pyrocystis* were the most frequently encountered (Fig. 9). *Gonyaulax*, *Triadinium*, *Podolampus*, *Ornithocercus*, *Ceratocorys*, *Prorocentrum*, *Dinophysis*, *Oxytoxum*, *Pyrophacus*, and *Zygabikodinium*, *Peridiniopsis* were found in lesser numbers. Luminescent dinoflagellates were represented in the genera *Ceratium*, *Protoperidinium*, *Pyrocystis*, and less frequently by cells of *Gonyaulax*, and *Ceratocorys*.

The tintinnids were represented by 19 genera; *Dictyocysta* sp. and *Eutintinnus* sp., the most abundant tintinnids were common to most of the samples in the transect.

Amphorella sp., *Steenstrupiella* sp., and *Dadayiella* sp. were also found in most samples but less frequently.

Of the crustacean plankton present in the samples, only *Pleuromamma* spp. copepods, euphausiid furcilia, and halocyprid ostracods proved to be luminescent when

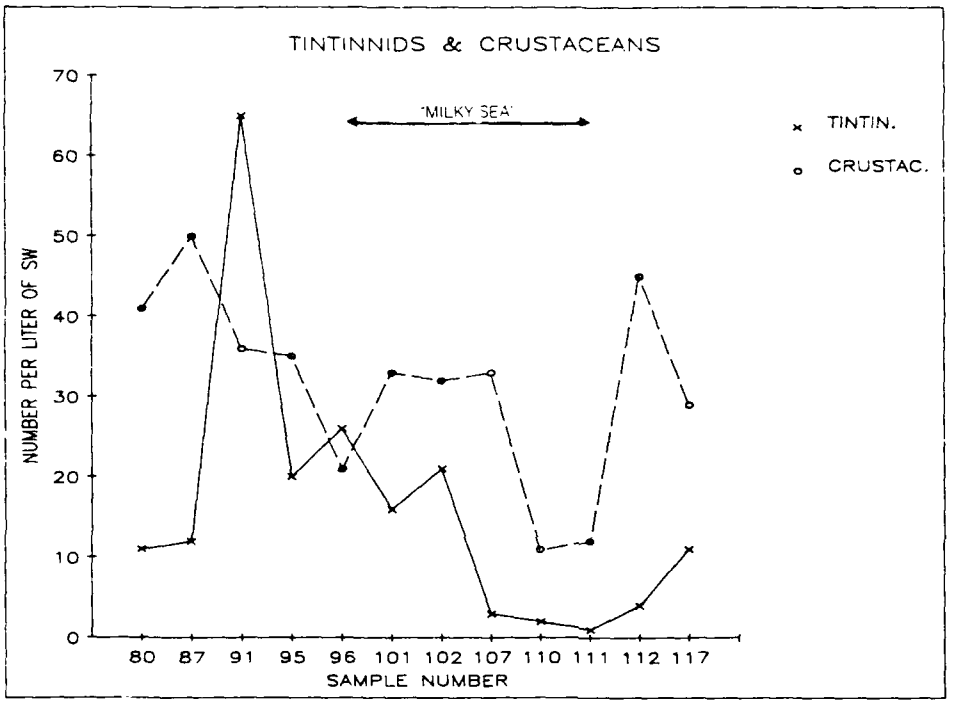


Fig. 8. Number of both tintinnids and copepod crustaceans (adult and larval stages) found per liter of seawater in plankton samples collected before, during, and after observed milky sea.

tested in this study. Other light emitting plankton found were siphonophore nectophores, radiolarians, and larvaceans. The numbers of dinoflagellates and zooplankters in the pumped samples have been extrapolated to $\cdot m^{-3}$ (Figs. 9,10).

On 24 July, the retrieval of a drifting plankton net (53 μm) tow (5 m to surface) revealed numerous colonies. They were free-floating although they were sometimes clumped and associated with other planktonic debris. These colonies appeared as tubes, spheres, and sheets, and they ranged in size from 0.5 to 1 mm but were occasionally as long as 10 mm. Some were photographed and later identified as *Phaeocystis* from preserved specimens (F. M. H. Reid, pers. comm.), a brownish-green biflagellate cell which develops into colonies (Fig. 11a,b). Extensive mucilaginous capsules form around the dividing cells which bind them together into large gelatinous clumps and can impart, at times, a slimy consistency to the ocean (Wimpenny, 1938; Tait & DeSanto, 1972).

In the isolation and testing of the colonies for bioluminescence in the LPTC, four samples displayed a continous light emission or glow characteristic of bacterial bioluminescence. This steady glow was observed and recorded for at least 1000 s on a multichannel analyser from each of the samples. In one of the samples (No. 101), 20

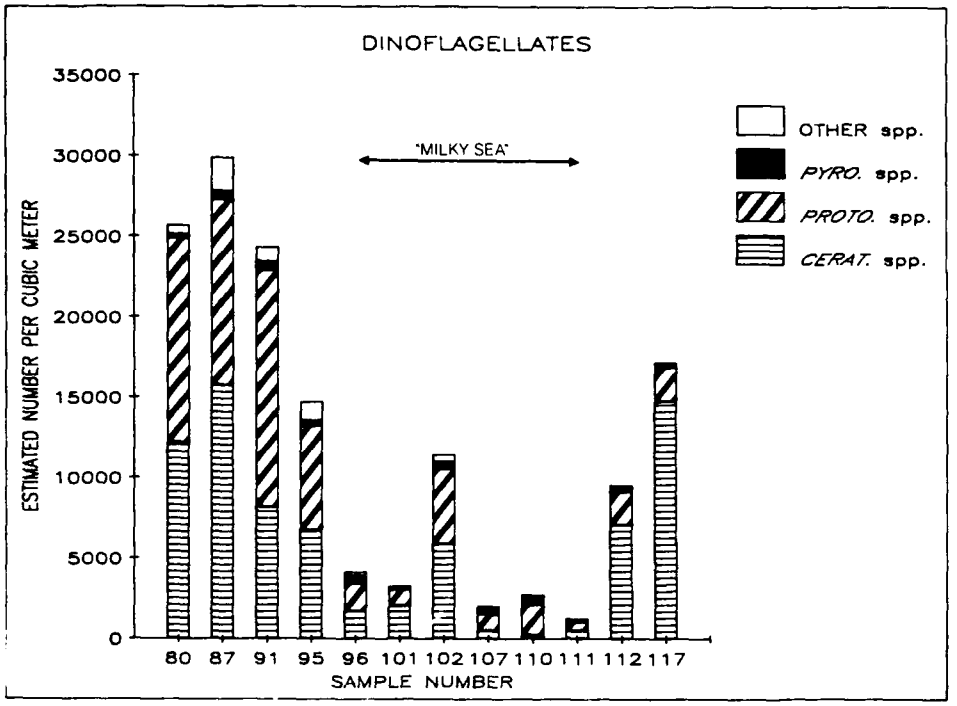


Fig. 9. Species of bioluminescent dinoflagellates and their estimated abundance found before, during, and after observed milky sea.

1000-s scans were recorded during 10 h of observations. The light emission rate of the glows among the samples ranged from $\approx 4 \times 10^6$ to 1×10^8 photons per second, (Table I). With these observations, colonies again were isolated from the same tow (7–24–1) and placed directly on agar media, streaked to distribute the material, and incubated at room temperature (25 °C). Two luminous colonies were observed on one plate and a third luminous colony was observed on another 21.5 h later (Fig. 12). The remaining surface area of all six plates were blanketed with other nonluminous colonies.

Plankton net Haul 7–24–1 collected organisms and debris from ≈ 5 m below and through the sea surface. The sample was placed in a 1-l beaker and observed to glow continuously for at least 2 days in a dark room although with diminishing intensity. A mottled glow was associated with the suspended debris, while a second glowing layer at the bottom of the beaker was associated with settled debris. Some of the glowing point

sources appeared brighter than others. Swirling and tapping of the glowing beaker evoked several intense short flashes at the surface of the beaker. When the beaker was examined under room lights, cells of the luminescent dinoflagellate *Pyrocystis* and small

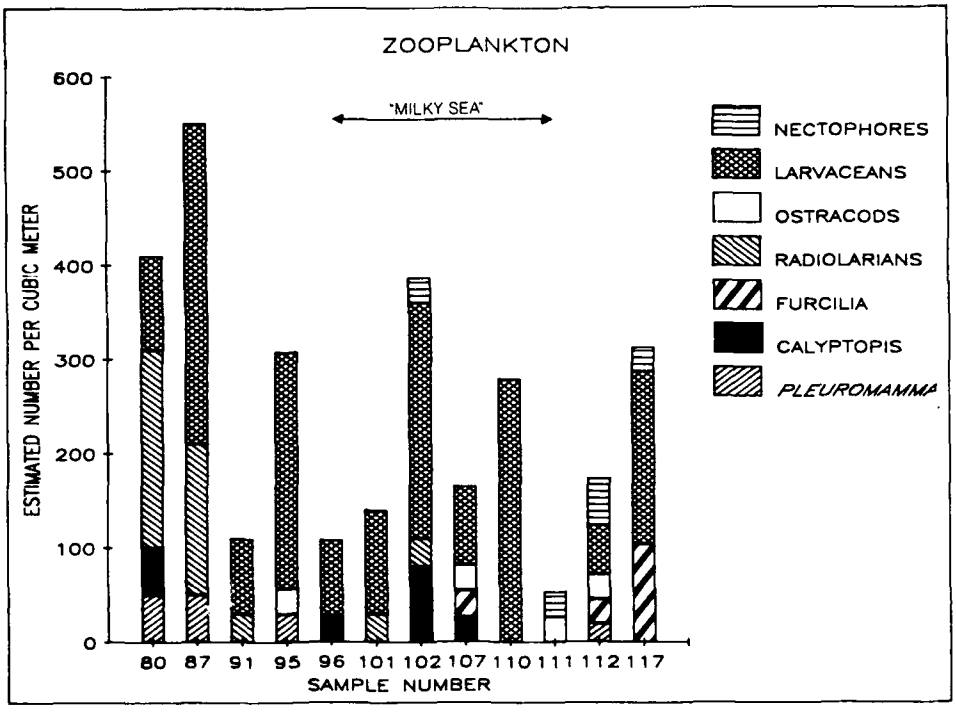


Fig. 10. Bioluminescent zooplankton and their estimated abundance found before, during, and after observed milky sea.

TABLE I

Field and laboratory history of luminous colonies.

Sample	Time/date	Position	Methods	Photons · s^{-1}
17	0333/24 July 1986	11°5.7′N : 59°3.5′E	2-l niskin bottle 5 m	4×10^6
101	0333/24 July 1986	11°5.8′N : 59°1.1′E	53-μm plankton net 5–0 m	2×10^7
112	0128/25 July 1986	12°18.3′N : 59°39.5′E	53-μm plankton net 5–0 m	1×10^8
157	0236/26 July 1986	15°02.2′N : 61°16.8′E	53-μm plankton net 5–0 m	6×10^6
Agar tube	0241/27 July 1986	–	Isolated from SWC agar plate, transferred to	3×10^8
Agar tube	2103/28 July 1986	–	agar tube	2×10^8

Fig. 11. (a) Photomicrograph of *Phaeocystis*, brown-green alga, collected in the study area with 53-μm net (10 × objective). (b) Close up view of *Phaeocystis* showing algal cell (AC) clusters imbedded in gelatinous matrix of colony (40 × objective).

Fig. 12. Epifluorescence photomicrograph of section of *Phaeocystis* with colonizing bacteria. Some bacteria encircle algal cells (larger spheres) where others appear clumped (AC, algal cell; B, bacteria; BC, bacteria clump; 100 × neofluar oil-immersion objective). Photomicrograph shows portion of Sample 112 which contains ≈275 bacteria in area of 0.001 mm^2. Because other observed areas on the colony had similar bacterial densities, entire surface of colony would contain $\approx 6 \times 10^6$ bacteria. Assuming that bacterial growth is surface-limited, bacterial concentration would approximate 6×10^8 bacteria per milliliter which is considered minimum because loosely associated bacteria would have fallen free during collection. Luminous bacteria account for only small fraction of general bacteria population.

swimming euphausiid furcilia were observed at the surface. Both are highly luminescent, however, their numbers were not sufficient to explain the intensity or the duration of the continuous light emission. Both *Pyrocystis* and furcilia produce intense flashes of short duration (100 s of milliseconds to seconds) compared with the continuous glow of luminescent bacteria (Fig. 13).

After 3 days of observations, the formerly glowing sample was filtered through a 10-μm collection cup to remove colonies, plankton, and debris from the filtrate. The particulate-free filtrate yielded numerous luminous colonies 24 h following SWC plate inoculation which supports the presence of a light emitter < 10 μm which can be bound or nonbound to the *Phaeocystis* colonies.

Of the 18 luminous isolates recovered, all were identified as *Vibrio harveyi* based on the nutritional properties tested (Reichelt & Baumann, 1973; Nealson, 1978). This uniformity among the isolates strongly suggests that a bloom of this species has occurred. Among its other properties, *V. harveyi* is a warm-water species and possesses a variety of extracellular enzymes that aid it in its existence as a saprophyte (Nealson 1978; Nealson & Hastings, 1979).

BIOLUMINESCENCE IN PLANKTON

Plankton collected from the onboard system (20-μm mesh porosity collection cups), surface net tows (53-μm mesh porosity net and collection cup), and from vertical 400–0-m tows (1/2 m ring, 500-μm mesh porosity net, and collection cup) were tested for light output in the LPTC. The plankton from deep tows were included in the testing

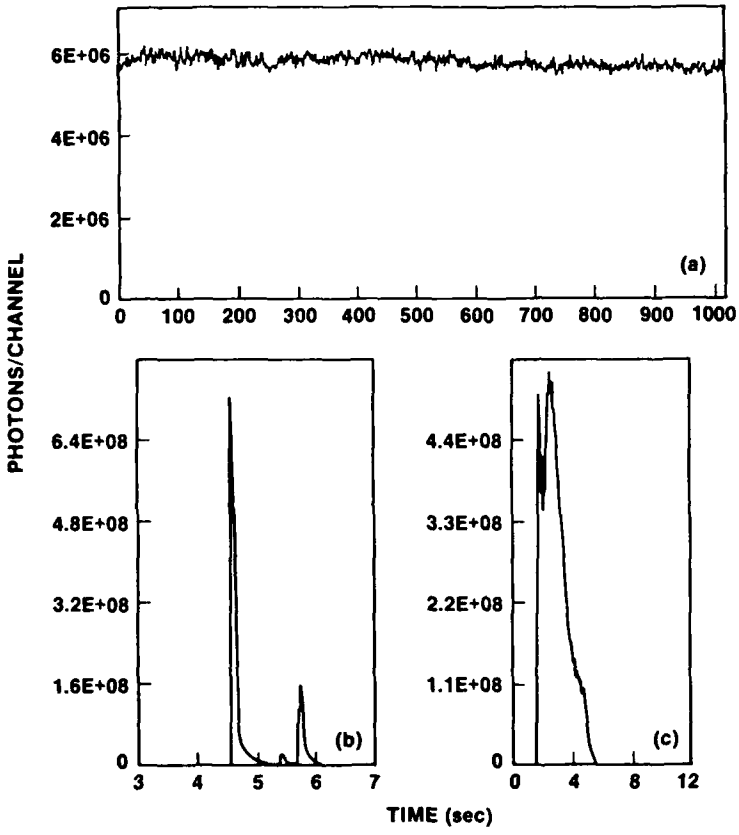


Fig. 13. (a) Light output (continuous glow) recorded from *Phaeocystis* colony colonized by luminous bacteria *Vibrio harveyii* recorded in LPTC; 1022 channel scan 1 s · ch^{-1}. (Sample 157, 26 July 1985). (b) Light output from single dinoflagellate cell *Pyrocystis noctiluca* recorded in LPTC; 10-s scan 10 ms · ch^{-1} which emitted light observed between Second 4 and 6 (from Tow 8, drifting 53-μm plankton net from 5 to 0 m; position: 7°24′N : 52°26′E, 15 July 1985). (c) Light output from single euphausiid furcilia larvae recorded in LPTC; 40-s scan 40 ms · ch^{-1} with emitted light observed between Second 1 and 6 (from Tow 3, vertical haul, 53-μm net, 30–0 m; position: 4°01′N : 51°01′E; 12 July 1985).

process to assess their light output and potential impact on the construction of a surface-water light budget. Plankton Tows 15 (10°05.462′N : 56°41.773′E) and 16 (8°56.294′N : 57°40.381′E) were collected near, but southwest of the milky sea, while Tow 17 (15°02.097′N 61°17.003′E) was collected in the milky sea. The plankton

composition and abundance of these tows are listed in Table II. From the plankton found in the onboard and net-tow collections, a bioluminescence light budget was estimated using average light output values (Table III) for various taxa together with the abundance of these taxa. The brightest flashes were produced by copepod species of *Pleuromamma* (*P. quadrungulata*, *P. gracilis* Claus, *P. abdominalis* Lubbock, and *P. xiphias* Giesbrecht), as did euphausiid furcilia, the dinoflagellate *Noctiluca miliaris*

TABLE II

Plankton composition of vertical net tows (500-μm mesh net) collected adjacent to and in milky sea.

Plankton	Sum of tows	Tow		
		15	16	17
		Total · 10 m^{-3}	Total · 10 m^{-3}	Total · 10 m^{-3}
Pyrocystis	319	128 (9)	111 (8)	80 (6)
Ostracods	261	87 (6)	73 (5)	101 (8)
Radiolarians	244	73 (5)	141 (10)	30 (2)
Furcilia	195	72 (5)	94 (7)	29 (2)
Pleuromamma	165	77 (6)	70 (5)	18 (1)
Euphausiids	90	39 (3)	15 (1)	36 (3)
Nectophores	58	22 (2)	21 (2)	15 (1)
Calyptopis	50	43 (3)	3 (<1)	4 (<1)
Tomopteris	2	0	0	2 (<1)

TABLE III

Light budget hierarchy for plankton tested in northern Indian Ocean and Arabian Sea in July 1985. Light output values were generated from single flash in LPTC and do not necessarily represent total light output available from single plankter.

Species/group	Photons · $flash^{-1}$	
	Mean	Range
Pleuromamma spp. copepods[a]	6×10^{10} (52)[c]	2×10^{7}–4×10^{11}
Euphausiid furcilia	3×10^{10} (17)	9×10^{7}–2×10^{11}
*Noctiluca miliaris**	1×10^{10} (2)	8×10^{9}–2×10^{10}
Halocyprid ostracods	9×10^{9} (15)	2×10^{7}–4×10^{10}
Siphonophores[b]	6×10^{9} (41)	3×10^{6}–1×10^{11}
Pyrocystis spp.*	5×10^{9} (21)	2×10^{7}–3×10^{10}
Radiolarians	3×10^{9} (12)	4×10^{7}–2×10^{10}
*Ceratocorys horrida**	3×10^{9} (3)	6×10^{7}–4×10^{9}
Larvaceans	2×10^{9} (12)	3×10^{6}–7×10^{9}
Protoperidinium spp.*	1×10^{9} (7)	7×10^{8}–4×10^{9}
Ceratium spp.*†	7×10^{7}	2×10^{7}–2×10^{8}

* Dinoflagellate species. † Found but not tested in this study; values for *Ceratium* from Sea of Cortez and Norwegian Sea used for same species found in this study. [a] Average light output for *P. quadrungulata*, *P. gracilis*, *P. abdominalis*, and *P. xiphias*. [b] Average light output from nectophores of *Diphyes*, *Hippopodius*, *Abylopsis*, and *Chelophyes*. [c] Number of plankters emitting light in this study.

(Suriray *ex* Lamarck), and halocyprid ostracods. The *Ceratium* dinoflagellates are among the weakest of emitters producing $\approx 10^7$–10^8 photons per flash. *Pyrocystis* and *Protoperidinium* dinoflagellates, siphonophore nectophores, radiolarians, and larvaceans emitted light at an intermediate level. The intensities within taxa ranged over several orders of magnitude (Table III).

The dinoflagellates dominated the light output per liter of seawater before and in the milky sea. In 12 samples examined, sufficient numbers of luminous dinoflagellates were present to produce > 50% of the estimated light output (Fig. 14). The samples prior to

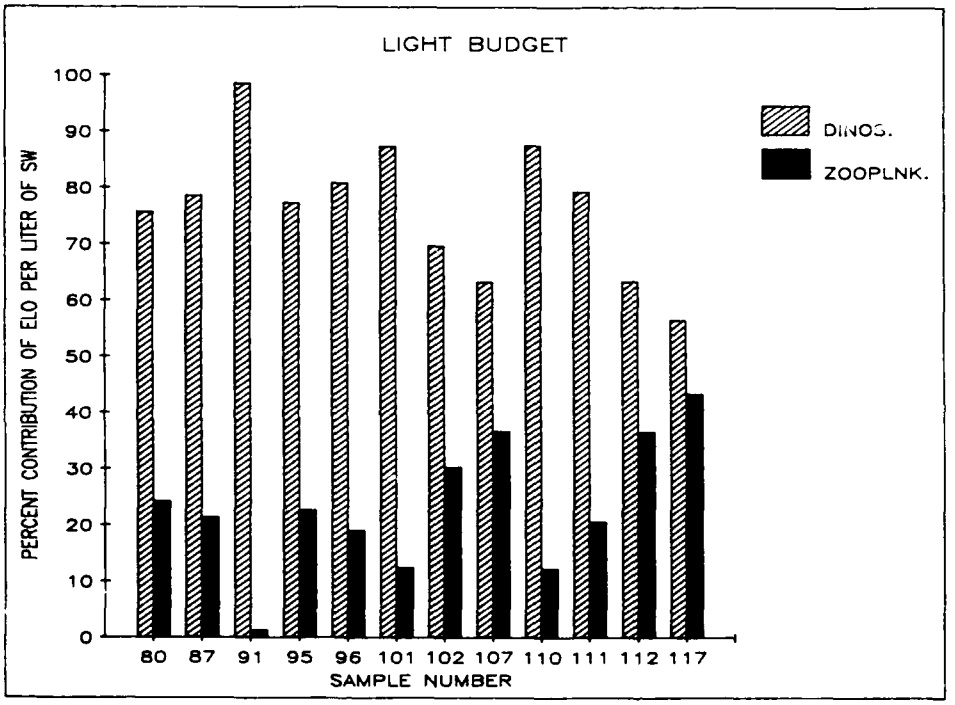


Fig. 14. Percent contribution by luminous dinoflagellates and zooplankton to estimated light output (ELO) per liter of seawater for plankton samples examined.

the milky sea were dominated by luminous species of *Protoperidinium*; however, once in the milky sea, both *Pyrocystis* and *Protoperidinium* contributed equally to the light output per liter of seawater (Fig. 15). *Ceratium* spp., *Ceratocorys horrida* (Stein), and *Gonyaulax* spp. did not contribute significantly to the overall budget.

Luminous zooplankters were present in all samples. Halocyprid ostracods, *Pleuromamma* copepods, and euphausiid furcilia were the dominant light-emitting zooplankton found in the onboard underway system samples (Fig. 16).

The identified and enumerated plankton from the pumped samples were multiplied by their respective light output values (Table III) per luminous plankter and were then

plotted as the summed estimated light output (SELO) from plankton per liter of seawater (Fig. 17). The bioluminescence intensity rate data from the onboard system were plotted at the time the plankton samples were collected. Through this reconstruction of the light budget, we observed similar bioluminescent activity trends for both systems. Similar peaks and valleys were observed between what was directly measured by the onboard system and bioluminescence activity reconstructed from the LPTC data.

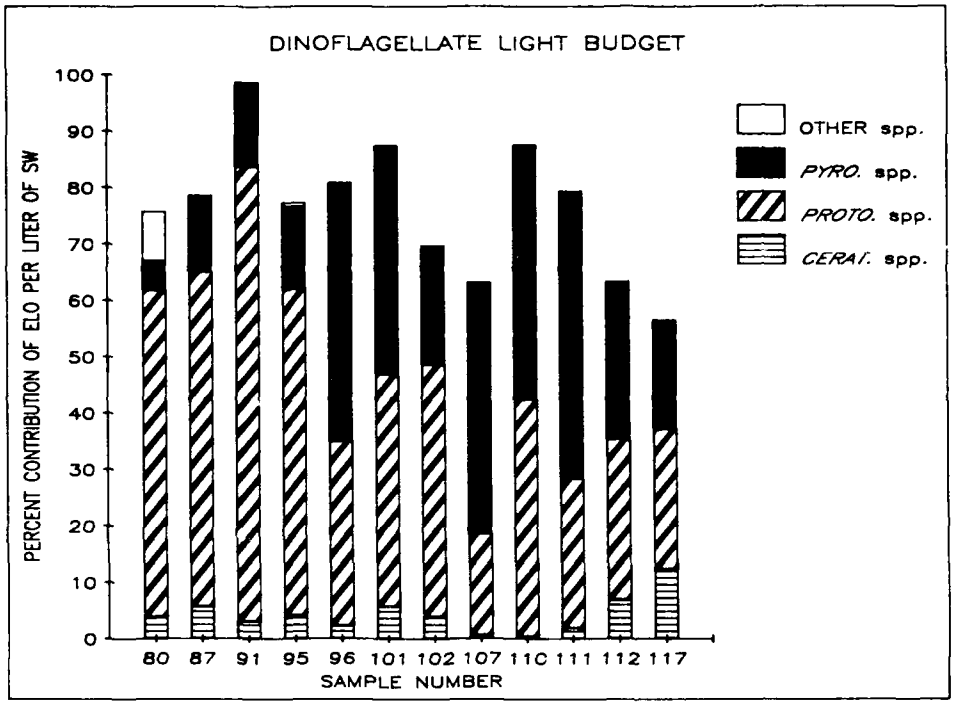


Fig. 15. Percent contribution by dominant luminous dinoflagellate species to estimated light output (ELO) per liter of seawater for plankton samples examined.

Discussion

In our studies, plankton assemblages changed throughout the transect. Diatoms and dinoflagellate numbers decreased entering and increased exiting the milky sea while total zooplankton numbers did not reflect an increase either before or after. Yet there was an overwhelming visual display of bioluminescence for three successive nights which implies that we have encountered a dual bioluminescence display. That is, a continuous light emission produced by *Vibrio harveyi* in conjunction with stimulable bioluminescence produced by dinoflagellates and zooplankton.

There are strong similarities between our observations and that reported by a ship transiting from Oman to Mombassa in August 1981 adjacent to our study area. White water was observed to spread out to the horizon as the ship entered the area with observations of sailing on top of a cloud and "although the wind remained force 8 the

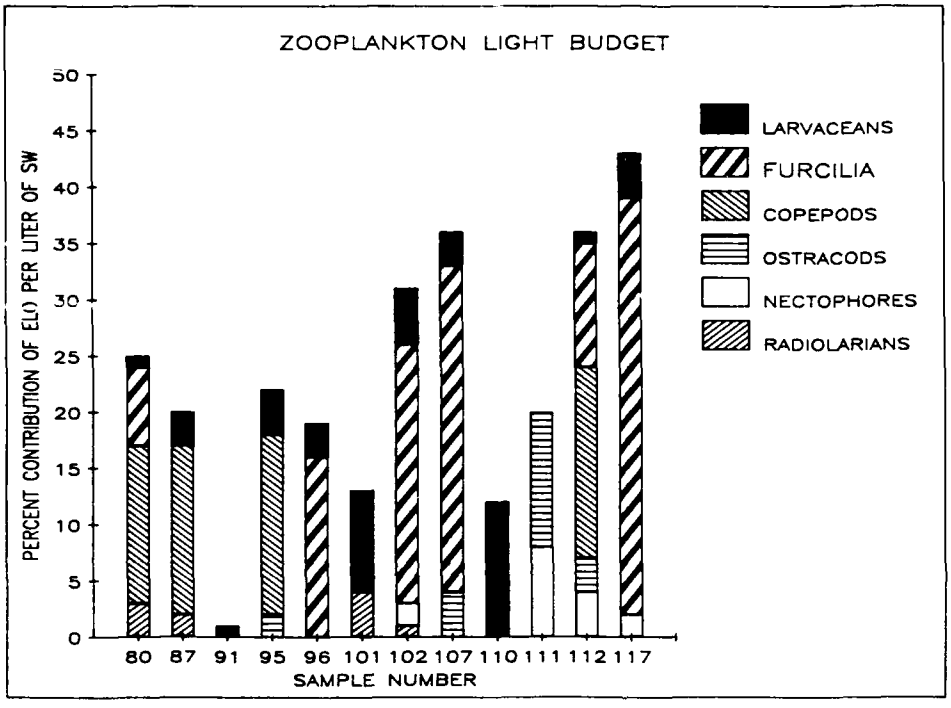


Fig. 16. Percent contribution by luminous zooplankton to estimated light output (ELO) per liter of seawater for plankton samples examined.

wave tops disappeared as if a light film of oil had been spread over the surface" (Anonymus, 1982, pp. 135–136).

Our onboard system bioluminescence measurements before and after the milky sea indicated little difference from that observed in the milky sea. High-time resolution scans recorded in the milky sea are of flash assemblages typical of dinoflagellate and zooplankton bioluminescence. A diel variation in bioluminescence intensity was maintained throughout the entire transect. Diel variation in bioluminescent-flash rate and intensity have been observed repeatedly in dinoflagellate populations (Sweeney *et al.*, 1959; Backus *et al.*, 1961; Seliger *et al.*, 1962; Yentsch *et al.*, 1964; Kelly, 1968; Losee & Lapota, 1981). The rate of luminescent flashing is a function of the sensitivity of photosynthetic dinoflagellates to light inhibition controlled by an endogenous rhythm in natural populations (Kelly & Katona, 1966). Vertical migration of bioluminescent organisms also has been observed as responsible for changes in bioluminescent intensity

throughout the water column (Seliger *et al.*, 1961). As total bioluminescent dinoflagellate cells decreased throughout the milky sea, there was a persistent population of *Pyrocystis* and *Protoperidinium* dinoflagellates. There is little difference between the numbers of *Pyrocystis* either outside (225–525 cells $\cdot$ m^{-3}) or within the milky sea

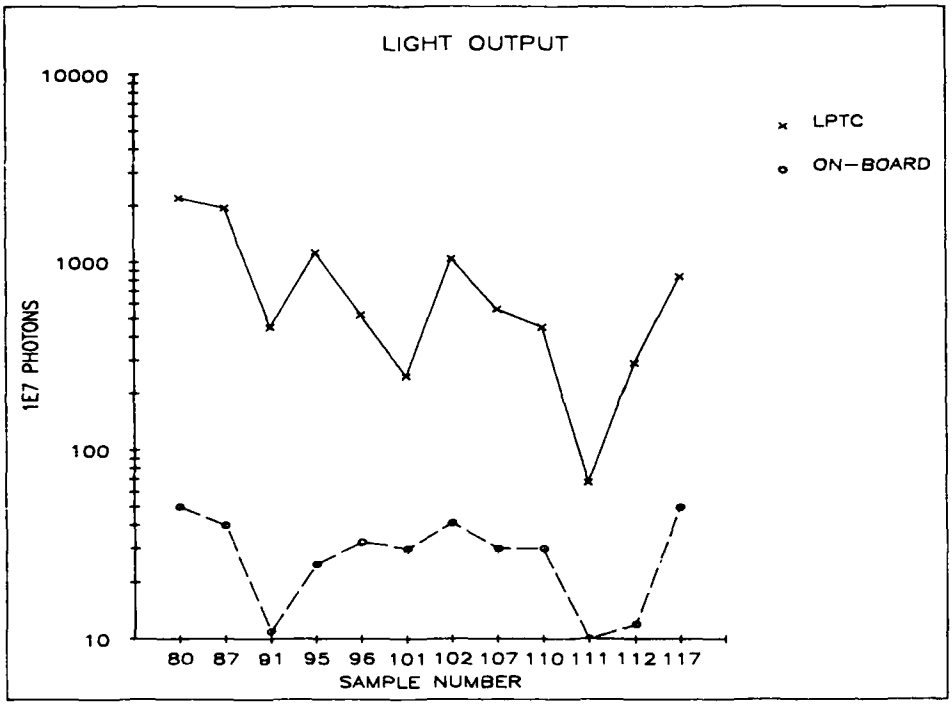


Fig. 17. Comparison of summed estimated light output (photons) from all luminescent plankton per liter of seawater and bioluminescence signal rate (photons per second per cubic centimeter of seawater) from onboard photometer system when plankton samples were collected.

(200–500 cells $\cdot$ m^{-3}) when compared with *Protoperidinium* and *Ceratium* (Fig. 9). *Pyrocystis* and *Protoperidinium*'s contribution to the dinoflagellate (Fig. 15) and overall (Fig. 14) bioluminescence light budget is significant. Stimulated bioluminescence of the heterotrophic dinoflagellate *Protoperidinium depressum* has been observed to decrease in intensity upon exposure to light (Tyul'kova & Filimonov, 1981), however, a diel bioluminescence rhythm is unknown in natural populations of *Protoperidinium*.

The luminous ostracod *Pyrocypris* was reported at times in dense concentrations off the east coast of India (Harvey, 1952) and 200 km off the coast southwest and northwest of Bombay, which exhibited an intense and continuous luminescence over a very large area (Tett & Kelly, 1973). Their tow samples revealed large quantities of this ostracod. Our plankton samples, either pumped (Fig. 10) or from net tows (Table II) taken inside

and outside the milky sea, do not reflect an unusual abundance of any zooplankters which might be responsible for the unvarying bioluminescence intensity of the sea.

The major zooplankton contributors to the bioluminescence light budget were copepods and larval euphausiids, although the luminescent larvacean *Oikopleura rufescens* Fol was commonly found in the pumped samples. Its light output is significantly less in a single flash than in *Pleuromamma* and the euphausiid furcilia when tested in the LPTC (Table III). Literature reviews (Harvey, 1952; Herring, 1978, 1985) on *Pleuromamma* copepods revealed that bioluminescence has been reported in *P. xiphias* (Clarke *et al.*, 1962; Rudjakov & Voronina, 1967), *P. indica* (Rudjakov & Voronina, 1967), *P. abdominalis* (Rudjakov & Voronina, 1967), *P. gracilis* (Rudjakov & Voronina, 1967; Evstigneev, 1983), *P. robusta* (Clarke *et al.*, 1962), and *P. piseki* (Evstigneev, 1983), but not in *P. quadrungulata*. Our observation identifies a seventh bioluminescent species in this genus and also confirms observations of bioluminescence made in *P. gracilis*, *P. abdominalis*, and *P. xiphias*. Bioluminescence was also measured for the first time in nectophores of three Calycophorae siphonophores. *Chelophyes contorta* is the second species observed to be bioluminescent in the family Diphyidae, the other identified as *Diphyes* (Harvey, 1952; Herring, 1978). We also observed bioluminescence for the first time in nectophores of *Abylopsis tetragona* and *A. eschscholtzi* in the family Abylidae. The only other mention of luminescence in this family was reported in *Abyla pentagona* from the Bay of Naples (Harvey, 1952).

Other studies have examined major contributors to the overall bioluminescence light budget in the Sargasso and Caribbean Seas (Swift *et al.*, 1983, 1985a,b). The dinoflagellate *Pyrocystis noctiluca* Murray *ex* Haeckel was estimated to produce 5–30% of the measured bioluminescence in the Sargasso Sea with most of the luminescence produced by zooplankton (crustaceans, larvaceans, and colonial radiolarians). The contribution to our light budget by *P. noctiluca* ranged from 5 to 51%, however, the combined luminescence of all *Protoperidinium* species ranged from 18 to 80% of the total, making this group, at times, the major contributors to the bioluminescence light budget in our samples. *Protoperidinium* dinoflagellates have been observed to contribute to the bioluminescence budgets in the Norwegian, Greenland, and Beaufort Seas (Lapota & Losee, 1983; Lapota, 1987; unpubl. data). The numbers of *P. noctiluca* found in our samples were consistently greater (range: 200–500 cells $\cdot$ m^{-3}) than found in the Sargasso Sea study (range: 62–275 $\cdot$ m^{-3}) (Swift *et al.*, 1985a) which may account for the difference in overall light production. There are great differences in the total numbers of bioluminescent plankters which we found in our study (summation of Figs. 9, 10) compared with the total numbers found in the Sargasso Sea (Swift *et al.*, 1985a). Approx. 200–500 bioluminescent organisms $\cdot$ m^{-3} were reported for the Sargasso Sea study while our study reflected concentrations of anywhere from 1300–30450 bioluminescent organisms $\cdot$ m^{-3}. Fifty percent of our samples reflected numbers of bioluminescent organisms in the range of 11800–30450 $\cdot$ m^{-3} in the Arabian Sea during the southwest monsoon.

No nutrient data was collected during our study, yet phosphates, nitrates, and trace

metals which are critical to biological productivity are generally found in lower concentrations in the Sargasso Sea than the Arabian Sea (Sverdrup *et al.*, 1942). The Arabian Sea during the southwest monsoon season experiences upwelled nutrient-rich water in the euphotic zone which results in high organic production (Friedrich, 1969; Zeitzschel, 1973). Historically, primary productivity values are > 500 mg $C \cdot m^{-2} \cdot day^{-1}$ in our study area (Koblentz-Mishke *et al.*, 1970). In contrast, the Sargasso Sea is characterized by old surface water with very little nutrient influx and low productivity values of 50 mg $C \cdot m^{-2} \cdot day^{-1}$ (Friedrich, 1969). The zooplankton standing crop in the southwest Sargasso Sea is generally low and constant and is characteristic of oligotrophic waters that do not show nutrient replenishment for phytoplankton growth (Menzel & Rhyther, 1961).

In another set of bioluminescence measurements, with similar onboard underway instrumentation and calibration conducted in the Sargasso Sea, a different character in the diel bioluminescence intensity was observed in comparison to our Arabian Sea measurements. The maximum intensity rarely exceeded 1×10^8 photons $\cdot s^{-1} \cdot cm^{-3}$ seawater and only for a brief period at night. The overall average intensity at night was $\approx 1/2$ the maximum count with daytime averages rarely exceeding the internal noise of the detector which is indicative of minimal stimulable bioluminescence (M. Geiger, pers. comm.). In comparison, the Arabian Sea bioluminescence intensity was, at least, an order of magnitude greater and sustained during the night hours while the daytime intensity was, on the average, greater by about a factor of 20.

Yet, there are other differences in methodology which may have contributed to our observation of a dinoflagellate dominance in the light budget. While total numbers of bioluminescent organisms $\cdot m^{-3}$ is indicative of the different type of water we are sampling, the changing character of the plankton assemblages is also of great importance. The summed estimated light output (SELO) per liter of seawater is based not solely on the total numbers of plankters present, but on their respective light output per type of plankter as well. Light output values generated for each type of plankter (Table III) is based on the amount of light produced from a single flash which is less than that found in a plankter stimulated to light exhaustion (Biggley *et al.*, 1969; Hamman & Seliger, 1973; Swift *et al.*, 1985a,b). The difference in methodology in assessing "light budgets" is based on the fact that luminescent plankton which have been discharged from our onboard detector system have not necessarily exhausted their bioluminescence potential. Visual observations of the plankton-laden water draining through the plankton discharge tank and collection cup indicate a myriad of flashes generated from the associated turbulence. Total stimulable light from the plankton is not being measured in either the onboard system or the laboratory plankton test chamber. In fact, the average residual time within the onboard system chamber is only 40 ms while the residence time in the eddies can be as long as 300 ms (Losee *et al.*, 1985) which is short compared with the total time of a single flash (100 s of milliseconds to seconds (Lapota & Losee, 1984). With the philosophy of pumping more water and thus more plankton past our detector ($0.67\ l \cdot s^{-1}$), we sacrifice complete flash signatures of

many plankters. However if we compare the bioluminescent signal of the onboard system to the summed estimated light output from plankton per liter of seawater, we observed an average of 4.3% of the bioluminescence potential (range: 0.6–12.1%) of the reconstructed light budget which is comparable to an observed 10% (Swift *et al.*, 1985a).

The isolation, identification, and photometric measurements of *Vibrio harveyi*, and its association with *Phaeocystis* colonies collected at the sea surface, strongly indicates that the milky sea is a sea-surface phenomenon and that the milky sea was not sampled by the onboard system by virtue of the seawater-intake depth (Fig. 2). A model incorporating an algal bloom near a frontal feature (Fig. 3) could be tested based on these preliminary observations. An algal bloom of, perhaps, diatoms dies and leads to algal lysis with an ensuing bloom of *V. harveyi* and other bacteria. The released algal lipids form a surface oil slick (which might also explain the calmer sea state observed in the presence of near-gale force winds) with the luminous bacteria concentrated at the surface on *Phaeocystis*. *V. harveyi* is cultured on *Phaeocystis* within the lipid slicks, reaching sufficient numbers to induce their luminous systems which accounts for the continuous glow that characterizes the milky sea.

From this first study of the milky sea phenomenon, several techniques for further collection of plankton samples are recommended. The first would be to deploy a floating surface intake from the ship while on station to sample the surface components, use an appropriate intake mesh size to exclude more intense sources of bioluminescence emitters which might mask the bacterial bioluminescence, such as dinoflagellates, copepods, larval euphausiids, etc., and measure the intensity and spectral components of a milky sea with existing onboard measurement systems. The physical dimensions of the display might be approximated from direct measurements. If a white or luminous haze is observed above the milky sea, the exposure of SWC agar plates on deck to the mist and spray might possibly demonstrate airborne luminous bacteria following incubation. The documentation of the number of luminous bacteria in surface waters before, during, and after a milky sea is essential in assessing the concentration factor of the luminous bacteria to induce this phenomenon and it would also be informative to assay bacterial luciferase activity at the sea surface. Future expeditions to the area to study this phenomenon should incorporate similar instrumentation with frequent calibration to ensure meaningful results. The collection, testing, and identification of other surface dwelling luminous and nonluminous plankton found in the milky sea layer is still of paramount importance in defining the bioluminescence field.

The examination of plankton collections, together with the concurrent testing of individual plankters for light emission, are essential for the construction of a light budget hierarchy. With this tool, it is possible to identify not only the major and minor light contributors of these displays but to those in other oceans.

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